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Fetal Hemoglobin Expression in the Compound Heterozygous State for −117 (G→A) $^{A}\gamma$ HPFH and IVSII-745 (C→G) β+ Thalassemia: A Case Study

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We studied a family in which two inherited defects of the non-α-globin cluster segregate: Greek hereditary persistence of fetal hemoglobin (HPFH) and β-thalassemia. The compound heterozygote is a healthy man with 43% HbF, $^{G}\gamma/^{A}\gamma$ ratio (27:73) differing from that of 10 simple heterozygotes for the Greek HPFH (92:8), normal levels of total Hb (13.3 g/dl), and reduced HbA_2 levels comparing with the levels of β-thal heterozygotes for the same mutation. Molecular analysis of the β-globin genotype revealed the presence of the IVSII-745 (C→G) β+ RNA splice mutation in trans with the −117 G→A Greek HPFH. The β+ mutation was linked to haplotype VII and the Greek HPFH was associated with haplotype Ia. The father of the compound heterozygote carries the Greek HPFH in trans with the −158 C→T on the $^{G}\gamma$ promoter, which is linked with haplotype IV. He presented 13.5% HbF with a $^{G}\gamma/^{A}\gamma$ ratio 75:25. His daughter was a compound heterozygote for the IVSII-745 mutation in trans with the −158 C→T, while her HbF levels were 3.7% with a $^{G}\gamma/^{A}\gamma$ ratio 31:69. Am. J. Hematol. 61:139–143, 1999. © 1999 Wiley-Liss, Inc.

Key words: hereditary persistence of fetal hemoglobin (HPFH); beta-globin mutation; thalassemia; haplotype; Xmn I

INTRODUCTION

Nondeletional hereditary persistences of fetal hemoglobin (ndHPFH) are benign conditions characterized by continuing expression of fetal haemoglobin (HbF) in adulthood. Sixteen point mutations producing the nd-HPFH phenotype have been defined in the last few years by sequencing analysis. Among them 11 are identified at the $^{A}\gamma$ gene promoter ($^{A}\gamma$-HPFH) and the remaining five at the $^{G}\gamma$-gene promoter ($^{G}\gamma$-HPFH) [1–2]. In the Greek type HPFH [3–6], it has been shown [7] that the (G→A) substitution at position −117 adjacent to the distal CAAT box of the $^{A}\gamma$ gene promoter is responsible for the $^{A}\gamma$ globin overexpression, possibly by inhibiting binding of a repressor [8]. In addition, as mentioned before [9–11], the activity of the HPFH β-globin gene is significantly decreased relative to normal; in fact the HPFH β-globin appears to be only 60–65% as active as a normal β-globin gene. Increased HbF levels have been associated with certain β-globin gene promoter mutations such as those in the proximal CACCC box [12,13], with the coinheritance of a high persistence of HbF determinant in cis to the mutated β gene as in the Sardinian δβ-thal [14]. We describe here a healthy Atheniau adult carrying −117 (G→A) Greek HPFH and β-thalassemia IVSII-745 (C→G) β+ in trans. IVSII-745 is not a very common mutation in Greece since it accounts only for the 6.9% of the total mutations causing β-thalassemia [15]. It was previously demonstrated [16] that this mutation is linked only to haplotype VII β-thalassemia chromosomes. The C→G substitution at IVS-2 position 745 in the gene of haplotype VII generates a new CAGGT sequence that serves as a new and deleterious internal splice site in the β-RNA precursor [17]. It is noteworthy that γ-globin expression is above 40% in this hitherto undescribed genetic condition, probably reflecting intensified stimulation of the mutated γ-promoter.

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TABLE I. Hematological Data and Biosynthetic Findings of the Members of the Family

Subjects	Age (years)	Sex	RBC 10^9/ml	MCV fl	MCH pg	Hb g/dl	% HbA_2	% HbF	% $^{A}\gamma^{I}$	% $^{G}\gamma$	% $^{A}\gamma^{T}$	β+γ/α ratio
I-1	59	M	4.7	79.4	24.2	11.4	1.4	13.5	75	25	0	0.85
I-2	55	F	5.6	69.7	21.8	12.4	4.6	0	0	0	0	0.41
II-1	32	M	6.2	67.4	21.5	13.3	3.7	43	73	27	0	0.68
II-2	27	F	5.5	71.3	23.1	12.2	4.9	3.7	31	69	0	0.50

TABLE II. Molecular Profile of the Members of the Family

	HPFH/β	Haplotype	$^{A}\gamma$ promoter–4-bp del	Xmn I site
Father	−117 $^{A}\gamma$HPFH/β^{A}	IV/Ia	−/−	+/−
Mother	N1/β°IVSII-745	IV/VII	−/−	−/−
Propositus	−117 $^{A}\gamma$HPFH/β°IVSII−745	VII/Ia	−/−	−/−
Sister	N1/β°IVSII-745	IV/VII	−/−	+/−

MATERIALS AND METHODS

Subjects

The propositus and his sister were discovered during population screening for thalassemia. When we discovered their molecular features, their parents were called for DNA analysis of the β-globin gene cluster. Hematological data were obtained by standard methods. In vitro globin chain synthesis was carried out on peripheral blood reticulocytes incubated with ^{3}H-leucine [18]; quantitation of HbF was done by cation-exchange high-performance liquid chromatography (HPLC) (Biorad/Variant™, California, USA). The column used was a 4.6 mm interior diameter (ID) by 30 mm. HbF elutes ahead and completely separates from HbA_{1c}. The sensitivity and accuracy of the method is 0.1% of HbF level. The $^{G}\gamma$, $^{A}\gamma^{I}$, and $^{A}\gamma^{T}$ were determined by the reversed-phase HPLC method, as previously described [20,21] and the column used was a 4.6 mm ID by 250 mm. DNA extraction, and Sutton's haplotype on the β-globin gene cluster were performed as reported for nine restriction fragment length polymorphisms (RFLPs) in the β-gene complex: Hinc II-ε, Xmn I-$^{G}\gamma$, Hind III-$^{G}\gamma$, Hind III-$^{A}\gamma$, Hinc II-ψβ, Hinc II-3′ψβ, Hinf I-5′β, Ava II-β, RSA I-3′β [22,23]. To determine the presence or absence of the 4-bp deletion 5′ to $^{A}\gamma$, $^{A}\gamma$, promoter DNA (−622 to +53) was amplified by polymerase chain reaction (PCR) [24]. The presence of the Greek HPFH mutation was determined by the denaturing gradient gel electrophoresis technique (DGGE) as previously described [25]. The C→T mutation at position −158 of the $^{G}\gamma$ gene promoter was studied by Xmn I digestion of DNA selectively amplified by PCR (from −623 to +28) and by DGGE analysis [25]. The presence of IVSII-745 was detected by amplification of a 577-bp fragment of DNA that includes a large part of IVS 2, exon 3, and a DNA fragment 3′ to exon 3 [12]. Amplified DNA was subsequently digested with the restriction endonuclease RSA I according to the manufacturer's guides.

RESULTS

The hematological and biosynthetic data of the Greek family are shown in Table I. The proband (II-1), a 30-year-old man, was discovered in an ongoing thalassemia screening program; he did not show anemia or splenomegaly. There were microcytosis and hypocromia; elevated HbA_2 level (3.7%), though lower than the mean value (4.7%) of 32 heterozygotes carrying only the IVSII-745 mutation; and increased HbF levels (43%). MCV, MCH, and total hemoglobin (67.4, 21.5, 13.3) were not different when compared with the group of male heterozygotes for the same beta-mutation (67.2, 21, 13.2). The mean value of HbF levels from 10 individual carriers of the −117 $^{A}\gamma$-HPFH was 13% so the proband shows a threefold increase of HbF associated with marked HbA reduction. Globin chain analysis of the HbF revealed that the proband presented different ratio of $^{G}\gamma$/$^{A}\gamma$ (27:73) from that measured (92:8) in 10 simple −117 HPFH heterozygotes. As red cell indices in the proband indicate a thalassemia heterozygote, we performed in vitro hemoglobin synthesis, which showed a significant reduction of β-globin chain production (β/α ratio 0.41) partially ameliorated by the high production of HbF (non-α/α ratio 0.68). The father (I-1) was a typical HPFH (Fig. 1b) with normal hematological indices and lower levels of HbA_2 (1.4%), which are in accordance with previously reported data [11]. The study of the Xmn I site was revealed positive (Fig. 1c), explaining in this manner the elevated Gγ levels (25%) found after the reversed phase HPLC separation, since it is already known that simple heterozygotes for the Greek type HPFH have essentially $^{A}\gamma^{I}$ globin chains [9].

The mother (I-2) was a simple IVSII-745 β-thalasse-

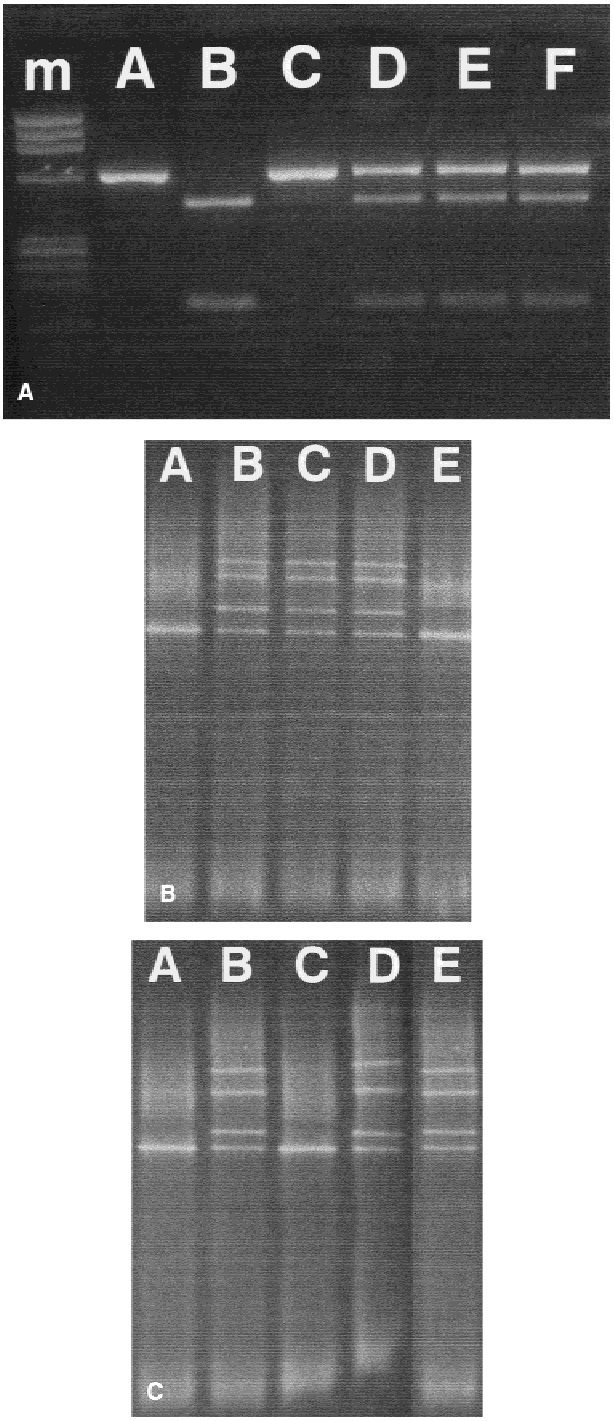

Fig. 1. (a) RSA I digestion of PCR product of the family members. A, control (nondigested of the mother's PCR); B, positive control of a homozygous for the IVSII-745 mutation; C, PCR product of the father; D, PCR product of the mother; E, PCR product of the son; F, PCR product of the daughter; m: ΦX174 digested with EcoR1. (b) DGGE analysis of the PCR product of the $^{A}\gamma$ globin gene promoter. A, PCR of the mother; B, positive control of a simple heterozygote for the −117 $^{A}\gamma$ HPFH; C, PCR of the father; D, PCR of the son; E, PCR of the daughter. (c) DGGE analysis of the PCR product of the $^{G}\gamma$ globin gene promoter. A, PCR of the son; B, PCR of the father; C, PCR of the son; D, PCR of the daughter; E, control of an individual with a T at −158 Gγ position.

mia heterozygote (Fig. 1a), with a C at −158 of the $^{G}\gamma$ promoter on both alleles and undetectable levels of HbF. The sister (II-2) was a simple IVSII-745 heterozygote with a T at −158 of the $^{G}\gamma$ promoter in one of the two alleles and 3.7% HbF. Genetic and molecular studies (Table II) showed that the −117 HPFH was linked to Hp I and the β° IVSII-745 to Hp VII in all cases. IVSII-745 was linked with a C at −158 of the $^{G}\gamma$ promoter, so the double heterozygote for the Greek HPFH and the thalassemic allele was negative for the Xmn I restriction site; his haplotype analysis revealed: VII/I (+−−−−−++−/+−−−−−+−−) . When we examined 10 heterozygotes for the IVSII-745 mutation the β-thal chromosome was never found associated with the Xmn I cleavage site. The members of the family were examined also for the 4-bp deletion (-AGCA) at −225 to −222 of the $^{A}\gamma^{T}$ promoter, which strongly correlates with decreased $^{A}\gamma^{T}$ expression [19] and, as was expected, they were found negative since it has been reported that the 4-bp deletion is associated with haplotype II.

DISCUSSION

HbF is the product of duplicated genetic loci. The $^{G}\gamma$ chain contains glycin at this position, while the $^{A}\gamma$ chain contains alanine. The $^{A}\gamma$ globin chain typically makes up approximately 30% of the γ-globin chains in newborns and approximately 60% in adults, and is therefore also involved in developmental switch [1]. It has been previously reported elevated HbF levels in compound heterozygous HPFH/β-thalassemia subjects [26–28]. The −117 (G→A) $^{A}\gamma$ HPFH mutation in Greeks is associated in vivo with 12–15% HbF levels in heterozygotes [5,6] and is linked to Hp I chromosome [5,29], whereas in Sardinians it is linked to Hp VII [11]. The same mutation was found in a Black family [40] associated with haplotype I, as occurs in Greeks. The −117 base change has been hypothesized to elevate γ-globin gene expression by decreasing binding of CP1 and CDP and increasing GATA-1 and NF-3 binding [30,31]. The −117 $^{A}\gamma$ HPFH and the IVSII-745 chromosomes in our compound heterozygote have C at −158 $^{G}\gamma$ (Xmn I negative) so that it can be excluded that the increase in $^{G}\gamma$ chain is due to the C→T substitution at −158. We do not know which of the two chromosomes is implicated in this increase in $^{G}\gamma$ synthesis. We think that it is more possible that the $^{G}\gamma$ gene in cis to the $^{A}\gamma$ HPFH is overexpressed by reason of the inhibitory effect of the $^{A}\gamma$ mutation, on the expression of the δ and β globin genes, than an Xmn I negative $^{G}\gamma$ gene in cis to the IVSII-745-thal locus. Similar results were previously published, where the −175 mutation in the $^{A}\gamma$-globin gene [34] is associated with a large increase of the expression of the adjacent $^{G}\gamma$ globin gene. On the other hand, the detected levels of HbF in the sister, who is a simple carrier of the mutated IVSII-745

determinant, could be attributed to the presence of the Xmn I site in trans with the β-thal mutation and probably to the presence of polymorphic sequences within the β globin gene cluster as mentioned before [32,33]. The −158 C→T mutation has been associated with increased HbF only under conditions of erythropoietic stress [36], although it has been demonstrated to increase F-cell numbers in normal blood donors [37].

The haplotype IV found to be associated with the normal β gene in this family accounts only for the 0.9% of the total haplotypes identified in Greece [38] and has been correlated with high HbF levels, with high $^{G}\gamma$ values, and the −158 $^{G}\gamma$ C→T mutation in patients with β-thalassemia [39]. The decreased activity of δ and β genes in cis to different ndHPFH [34] indicate a cis competition of the γ-globin gene cluster for the locus control region (LCR) 5′ to the ε-globin gene. We observed a 16% reduction of the δ-gene's expression and a much higher diminution of the β-gene's expression 40% in cis with the $^{A}\gamma$ mutation.

It has been recently demonstrated that in addition to the two factors (beta thalassemia and Xmn I-G gamma site) on chromosome 11p, there is a third major genetic determinant for fetal Hb production localized on chromosome 6q [35]. The molecular study of HPFH conditions in other families would provide us with useful data trying to understand the in vivo interaction of mutated γ and β genes with the fetal hemoglobin expression.

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